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(54) Title: IMPROVED RECOMBINANT PROTEIN PRODUCTION

(57) Abstract

In a method for effecting an increased expression in serum-free medium of recombinant proteins in a host cell being able to express said protein said host is cultured in a serum-free medium comprising an egg yolk fraction being free of lipoprotein and lipids so as to express said protein. Also the use of an egg yolk fraction being free of lipoprotein and lipids for increasing the expression of recombinant proteins in a host cell being able to express said protein in a serum-free cell growth medium is disclosed.

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IMPROVED RECOMBINANT PROTEIN PRODUCTION

TECHNICAL FIELD

This invention relates to a method for effecting an increased expression of recombinant proteins in serum-free media.

BACKGROUND OF THE INVENTION

A number of proteins having biological activity, e.g. blood coagulation factors such as Factor VII, Factor VIII, Factor IX, Factor X, and Factor XIII, immunoglobulins or serum albumin have traditionally been isolated from body fluids such as serum as their only source. However, as some of the proteins are only present in small amounts, a vast number of donors have to be involved in order to allow isolation of the proteins in industrial scale. This has given rise to increased concern due to the risk of transferring blood borne diseases which together with the limitation on the natural ressources has enhanced the search for alternative methods for producing such proteins in industrial scale without such risks.

Hemophilia A is an X-chromosome-linked inherited disease which afflicts 1-2 males per 10,000. The desease is caused by an absence of deficiency of Factor VIII:C. Factor VIII:C is a very large glycoprotein (native M_r 330 K - 360 K), which is present in plasma at extremely low concentrations. It is a necessary element in the proteolytic cascade which converts soluble fibrinogen to insoluble fibrin, forming a clot to prevent blood loss from traumatized tissue. In the bloodstream, it is found in noncovalent association with von Willebrand factor (vWF) which acts as a stabilizing carrier protein. Factor VIII:C is very susceptible to cleavage by thrombin, plasmin, activated protein C, and other serine proteases. It is generally isolated from plas-ma or plasma products as a series of related polypep-

tides ranging from M_r 160 K-40 K with predominant species of M_r 92 K and M_r 80 K-77 K. This complex pattern has made the analysis of the structure of active Factor VIII:C very difficult.

Recombinant proteins produced according to the invention may be Factor VII, Factor VIII, Factor IX, Factor X, and Factor XIII which may be used for substitution therapi in individuals having deficiencies in the coagulation cascade, immunoglobulin which may be used for treating infectious diseases or serum albumin which may be used as substitute for blood transfusion during operations or a constituent of a culture medium.

Recombinant proteins having Factor VIII:C activity being prepared according to the present invention may be full length Factor VIII:C corresponding to the protein isolated from plasma, or a derivative thereof having the capability of normalizing the insufficient blood clotting caused by deficiency of Factor VIII:C. The derivatives of Factor VIII:C may be shortened single chain forms or derivatives comprising two chains. Even fragments of Factor VIII:C which may not per se show coagulant activity, but which may be used in the treatment of haemophiliacs e.g. for saturation of antibodies against Factor VIII:C present in inhibitor patients.

The proteins produced in accordance with the present invention show homology with all or a part of the natural Factor VIII:C molecule.

The preparation of recombinant proteins having Factor VIII:C activity by recombinant techniques has inter alia been disclosed in a number of patent publications. Thus, European Patent Application No. 160 457 and International Patent Application No. WO 86/01961 disclose the production of full length Factor VIII:C, and European Patent Application No. EP 150 735, International Patent Application No. WO 86/06101, European Patent Application No. EP 232 112, International Patent Application No. WO 87/04187, International Patent Application No. WO 87/

07144, International Patent Application No. WO 88/00381, European Patent Application No. EP 251 843, European Patent Application No. EP 253 455, European Patent Application No. EP 254 076, U.S. Patent No. 4.980.456, European Patent Application No. EP 294 910, European Patent Application No. EP 265 778, European Patent Application No. EP 303 540, International Patent Application No. WO 91/07490, and International Patent Application No. WO 91/09122 disclose recombinant expression of subunits of Factor VIII:C or co-expression of subunits for the production of complexes showing coagulany activity or binding affinity to antibodies inhibiting Factor VIII:C.
Expression of recombinant full length human Factor VIII:C is usually low and the molecule is unstable due to proteolysis.

Derivatives of Factor VIII:C in the form of shortened single chain forms or derivatives comprising two chains have also been successfully produced by recombinant techniques. Although these derivatives normally are expressed in a higher yield than full-length Factor VIII:C, there is still a desire for increasing the level of expression.

In order to obtain an acceptable level of expression and an acceptable stability of a produced recombinant protein having biological activity it is often preferred to express the proteins in mammalian cells. These cells are usualy grown in media containing mammalian serum e.g. new borne or fetal bovine serum, in amounts of about 10% serum by volume relative to total media volume. The recombinant protein is secreted into the culture medium and has to be separated from the serum components during the isolation.

However, serum has high contents of proteins which renders the recovery and purification of the desired proteins troublesome. Such addition may also introduce a potential risk of introducing malign viruses from the bovine serum into the final blood preparations. Hence, there is a need to find serum-free culture media or media having reduced amounts of serum for culturing

the host cells without essentially reducing the expression or the stability of the desired protein.

Several methods of expressing proteins having biological activity in serum-free media has already been proposed.

WO 87/04187 and EP 251 843 disclose that expression of Factor VIII:C in serum-free medium in the presence of von Willebrand Factor (vWF) or phospholipide increases the expression of Factor VIII:C. In EP 254076 it is disclosed that addition of lipoprotein to a serum-free medium increases the expression of Factor VIII in a host cell carrying the gene encoding Factor VIII. JP 61 063283 discloses that expression of i.a. monoclonal antibodies in serum-freee media may be increased by addition of lipoprotein in the form of an egg yolk fraction. Finally, EP 441 695 discloses the expression of Factor VIII:C or an analogue thereof in serum-free medium in the presence of a cationic or anionic polymer, preferably a polysaccharide which most preferred is in a sulphatized form. It has been proposed to accelerate the growth of Myobacterium cultures or to stabilize media for cultivating tuberculosis bacilli by adding whole egg yolk.

The present invention is based on the surprising finding that a fraction of egg yolk not being freed from lipids may be used for increasing the expression of recombinant proteins having biological activity, preferably Factor VIII:C, in serum-free media.

DISCLOSURE OF THE INVENTION

The present invemtion relates to a method for effecting an increased expression, in serum-free medium, of recombinant proteins in a mammalian host cell being able to express said protein comprising culturing said host i a serum-free growth medium comprising an egg yolk fraction being free of lipopro-

tein and lipids so as to express said protein.

DETAILED DESCRIPTION OF THE INVENTION

It has surprisingly been found that the addition of an egg yolk fraction being free of lipoprotein and lipids increases the expression, in serum-free medium, of recombinant proteins.

The fraction of egg yolk used according to the invention may be produced by adding polyethylene glycol to a concentration of 3.5% and buffer to egg yolk and stir and centrifuge as disclosed in Immunological Communications, 9(5), 475-493 (1980). In this precipitation, the lipoprotein fraction of the egg yolk is precipitated. The lipoproteins are micelles comprising lipids having their lipofil parts internally and a surface cmprising i.a. apoproteins. The lipids are constituted by fatty acids phospholipids and cholesterol. The fraction to be used in accordance with the present invention is the supernatant from the first centrifugation not being a desired fraction according to the above reference dealing with the isolation of antibodies from the precipitate. There is no indication of any use of this supernatant in the reference.

In such separation, all constituents of the lipoproteins which have previously been proposed as supplement to increase the expression of recombinant proteins in serum-free media are isolated from the egg yolk, as a precipitate.

According to a preferred aspect of the invention, the egg yolk fraction is added to a concentration of from 0.5 to 15 %, a concentration of from 1 to 10 % being more preferred an a concentration of 5 to 10 % being most preferred.

According to preferred aspect, the invention relates to a method for effecting an increased expression of a protein

having Factor VIII:C activity. According to a more preferred aspect of the invention, the method is used for expression of a complex of the 92kD and 80/77kD subunits of Factor VIII:C. For such cultivation the egg yolk fraction does not only increase the level of expression of the individual subunits of Factor VIII:C, but also increases the degree of complex formation and stabilizes the produced complex and thus increases the yield of active product.

In another aspect, the invention relates to the use of an egg yolk fraction being free of lipoprotein and lipids for increasing the expression of recombinant proteins in a host cell being able to express said protein comprising culturing said host i a serum-free cell growth medium.

In still another aspect, the invention relates to the use of an egg yolk fraction being free of lipoprotein and lipids for increasing the expression of recombinant proteins having Factor VIII:C activity.

The term "Heavy Chain (HC)" is used to designate the 92 kD subunit of Factor VIII:C.

The term "Light Chain (LC)" is used to designate the 77/80 kD subunit of Factor VIII:C.

The term "co-expressing" as used herein in combination with proteins having Factor VIII:C activity refers to simultaneous expression of the 92 kD and the 80 kD subunits of Factor VIII:C within the same host cell. The polynucleotide sequences encoding the 92 kD and 80 kD subunits may be on the same or on different expression cassettes or plasmids. Co-expression of the 92 kD and 80 kD subunits permits proper folding to occur, which in turn provides a complex having activity and efficiency of secretion.

The term "cell growth medium" as used herein refers to any me-

dium suitable for culturing host cells, and includes media suitable for obtaining expression of recombinant products whether actual cell "growth" occurs or not. Cell growth media generally include nutrients and a metabolizable energy source in an aqueous solution. If desired, cell growth media may also include a compound which induces expression of the recombinant polypeptides of the invention. Selection of such an inducing compound depends upon the promoter selected to control expression. Other typical additives include selection compounds (i.e., drugs or other chemicals added to the media to insure that only transformed host cells survive in the medium).

The term "serum-free medium" as used herein is intended to designate a solution which has been supplemented to such an extent that the necessary trace factors present in serum need not be added in the form of serum. There are many suitable cell growth media available from commercial sources.

The term "egg extract" is used here to designate a fraction of egg yolk being free of lipoprotein and lipids prepared as described in Immunological Communications (ibid).

The structural genes typically include a leader sequence coding for the signal peptide which directs the polypeptide into the lumen of the endoplasmic reticulum for processing and maturation. Optionally included are additional sequences encoding propeptides which are processed post-translationally by endopeptidases, where the endopeptidases cleave a peptide bond, removing the propeptide to generate the mature polypeptide. The signal peptide may be the naturally occurring one, particularly for the N-terminal peptide, or may be any signal peptide which provides for the processing and maturation of the polypeptides.

Various mammalian host cells may be employed in which the regulatory sequences and replication system are functional. Such cells include COS7 cells, Chinese hamster ovary (CHO) cells,

mouse kidney cells, hamster kidney cells, HeLa cells, HepG2 cells, or the like, e.g VERO cells, W-138 or MDCK cell lines.

Proteins having Factor VIII:C activity produced according to the invention are primarily intended for treatment of hemophiliacs and patients suffering from other conditions involving blood clotting disorders. The subject proteins may be administered in physiologically acceptable carrier, such as water, saline, phosphate buffered saline, and citrate buffered saline, at concentrations in the range of about 10-200 U/mL. See U.S. Patent Nos. 3,631,018; 3,652,530, and 4,069,216 for methods of administration and amounts. Other conventional additives may also be included. They also have a variety of uses as immunogens for the production of antibodies, for isolation of von Willebrand factor by affinity chromatography and in diagnostic assays for Factor VIII:C.

The invention is explained more in detail in the below examples which provide guidance to the skilled art worker how to work the invention and are not to construed as limiting the scope of protection.

EXPERIMENTAL PART

MATERIALS AND METHODS

The cell growth medium used was prepared as follows:

Seromed mem-Dulbeco TZ 043C powder for 10 l was dissolved in 8.5 l Milli-Q-water.

The following was then added:

0.977 g $MgSO_4$ anhydrous

37.0 g $NaHCO_3$

6.2 g NaCl

1.5 g l-proline
1.1 g Na-pyruvate

After adjusting the pH to 7.4 using hydrochloric acid the volume was adjusted to 9 l by adding demineralized water. The solution was steril filtered.
When used, 10% 1 M aqueous NaCl was added.

A basis medium was then prepared by adding the following constituents:
2.5 g/l Papain digested soy protein
5 mg/l insulin
5.5 mM Gly-Gly
25 μM $Fe_2(SO_4)_3$
6.5 μg/l Sodium selenite
0.1 g/l Dextran MW 67000
0.5 g/l ϵ-ACA

EXAMPLE 1

PROVIDING CELL LINES CO-EXPRESSING FACTOR VIII:C HEAVY CHAIN AND LIGHT CHAIN

Transfection-procedure

The DHFR$^-$ CHO cell line DG44 (G. Urlaub et al., Som Cell Mol Genet (1986) 12:555-566) was first transfected with the plasmid pCMF8-80AT: In this plasmid the CMV promoter (described in example 7 of WO91/07490) transcribes the FVIII-LC cDNA derived from pSVF8-80AT (described in example 6) and downstream is placed the Ad-MLP/dhfr casette derived from pAd-DHFR (described in example 4 of WO91/07490). The transfection method used was the polybrene method of W. CHaney et al. (Som Cell Mol Genet (1986) 12:237-244). By selection of DHFR$^+$ cells (DMEM + 10% DFCS) several FVIII-LC producers were isolated; one of these was designated 11W.

In order to introduce FVIII-HC in 11W the cell line was cotransfected with the plasmid pPR78 (this plasmid is an analog to pCMVF8-80AT, but harbours instead of the FVIII-LC cDNA the FVIII-HC cDNA derived from pCMVF8-92R described in example 8 of WO91/07490) and pSV2-neo (P.J. Southern and P. Berg, J Mol Appl Genet (1982) 1:327-341). The transfection method used was the modified calcium phosphate procedure of C. Chen and H. Okayama (Mol Cell Biol (1987) 7: 2745-2752). Transfectants were isolated in medium containing 700 µg Geneticin (G418 Sulphate, Gibco) per ml. Cells from the primary pool were subcloned by the limited dilution method and the individual clones were tested for expression fo active FVIII. In this way several FVIII:C producing cell lines were isolated and one of these was designated "45".

The cells selected in this way on the basis of the expression level were seeded into T-flasks for cultivation in the absence of egg extract or in the presence of egg extract in various concentrations.

The description of transfection referred to in WO91/07490 is hereby incorporated by reference, including the reference to the plasmide pSVF8-92, pSVF8-80 and pSVF8-200 deposited under the accession number ATCC 40222, ATCC 40223 and ATCC 40190, respectively.

EXAMPLE 2

CULTURING CELL LINE CO-EXPRESSING FACTOR VIII:C HEAVY CHAIN AND LIGHT CHAIN

The results of cultivating in T-flasks as described above, are shown in the below Tables.

The following additions of egg extract were tested: 1%, 2.5%,

5% and 10% in basis medium. These were compared with the basis medium without addition of egg extract.

In Table 1 is stated the result of the determination of coagulant activity by Coatest kit (KabiVitrum), and in Table 2 and 3 the results of the determination of FVIII:HC and FVIII:LC, respectively.

Table 1

FVIII:C U/ML

DAYS	1 BASIS + 10% egg extr.	2 BASIS + 5% egg extr.	3 BASIS + 2.5% egg extr.	4 BASIS + 1% egg extr.	5 BASIS
3	1.120	0.58	0.549	0.47	0.310
5	2.778	1.48	1.300	0.97	0.725
7	3.947	2.77	2.280	1.99	1.470
10	8.440	5.44	4.430	4.08	2.920

Table 2

FVIII:HC U/ML

DAYS	1 BASIS + 10% egg extr.	2 BASIS + 5% egg extr.	3 BASIS + 2.5% egg extr.	4 BASIS + 1% egg extr.	5 BASIS
3	5.25	3.90	2.95	3.05	2.25
5	12.65	7.60	6.90	7.05	7.10
7	24.50	17.35	14.00	14.80	13.80
10	> 50	44.00	38.50	39.00	35.00

Table 3

FVIII:LC U/ML

DAYS	1 BASIS + 10% egg extr.	2 BASIS + 5% egg extr.	3 BASIS + 2.5% egg extr.	4 BASIS + 1% egg extr.	5 BASIS
3	18.10	13.6	14.0	13.87	12.60
5	34.50	22.0	25.0	25.57	22.25
7	49.00	38.5	33.5	32.09	32.50
10	109.50	99.5	79.5	68.08	62.00

CLAIMS

1. A method for effecting an increased expression of recombinant proteins in a mammalian host cell being able to express said protein comprising culturing said host i a serum free cell growth medium comprising an egg yolk fraction being free of lipoprotein and lipids so as to express said protein.

2. The method as claimed in claim 1 wherein the concentration of the egg yolk fraction is from 0.5 to 15%

3. The method as claimed in claim 2 wherein the concentration of the egg yolk fraction is from 1 to 10 %.

4.The method as claimed in claim 3 wherein the concentration of the egg yolk fraction is from 5 to 10 %.

5. The method as claimed in any of claims 1-4 wherein the recombinant protein produced is a protein having Factor VIII:C activity.

6. The method as claimed in claim 5 wherein the protein having Factor VIII:C activity produced is a complex of the 92kD and 80/77kD subunits of Factor VIII:C.

7. Use of an egg yolk fraction being free of lipoprotein and lipids for increasing the expression of recombinant proteins in a host cell being able to express said protein in a serum free cell growth medium.

8. Use as claimed in claim 7 for expressing a protein having Factor VIII:C activity.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 93/00138

A. CLASSIFICATION OF SUBJECT MATTER

IPC5: C12N 5/00, C12N 15/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC5: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information Services, file 351, WPIL, Dialog acc.no. 008053976, (QP CORP), "Nutritive compsn. of animal cell use - contg. granular fraction or water-soluble fraction of egg yolk, as main component", JP 1235586, A, 890920, 8944	1
Y		1-8
	--	
X	Journal of Cellular Physiology, Volume 114, 1983, D.K. Fujii et al, "Phosphatidyl Choline and the Growth in Serum-Free Medium of Vascular Endothelial and Smooth Muscle Cells, and Corneal Endothelial Cells" page 267 - page 278	1-4
Y		1-8
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[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
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Date of the actual completion of the international search	Date of mailing of the international search report
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International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Dialog Information Services, File 351, WPIL, Dialog acc.no. 008118174, (CHUKO KASEI KOGYO), "Serum-less culture of animal cell - has liposome with natural polysaccharide with prim. alcohol substd. with aminoethyl carbonyl gp.", JP 1289484, A, 891121, 9001	1
Y		1-8
	--	
Y	Dialog Information Services, file 155, Medline, Dialog acc.no. 05812414, Yuo S. et al: "Induction of macrophage growth by lipids", J Immunol Feb 15 1986, 136 (4) p1334-8	1-8
	--	
A	Biochimica et Biophysica Acta, Volume 710, 1982, A.O. Yau-Young et al, "Mobilization of cholesterol from cholesterol ester-enriched tissue culture cells by phospholipid dispersions" page 181 - page 187	1
	--	
A	Dialog Information Services, file 155, Medline, Dialog acc.no. 07265794, Ito H. et al: "Development of a serum-free medium and primary culture of human renal cell carcinomas by serum--free cultur", Nippon Hinyokika Gakai Zasshi Dec 1989, 80 (12) p1741-8	1
	--	
A	Dialog Information Services, file 155, Medline, Dialog acc.no. 05922710, Martis M.J. et al: "A simple fractionation of chicken egg yolk yields a protein component that stimulates cell proliferation and differentiation in primary avian tendon cells", In Vitro Cell Dev Biol May 1986, 22 (5) p241-6	1
	--	
Y	WO, A1, 8704187 (GENETICS INSTITUTE, INC.), 16 July 1987 (16.07.87)	1-8
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/DK 93/00138

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Dialog Information Services, File 350, WPIL, Dialog acc.no. 000826084, (ARAI K), "Dry egg culture medium prodn", JP 71036191, B, 7142 ---	1
Y	Dialog Information Services, file 351, WPIL, Dialog acc.no. 007871164, (TAIYO CHEMICAL IND KK), "Animal cell growth factor prepn. - by selectively removing yolk lipo-protein from yolk soln.", JP 1083100, A, 890328, 8918 --- --------	1-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

02/07/93

International application No.
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 8704187	16/07/87	DE-A- 3785102	06/05/93
		EP-A,B- 0253870	27/01/88
		US-A- 5198349	30/03/93